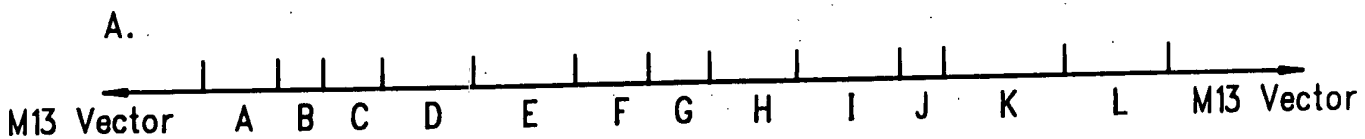


FIG. 1

DNA Sequences of Oligos used to delete CDRI-CDR3 regions of 668-4

Kappa Chain

Framework 4 ← | Stop | Stop | Stop | Framework 1 →

TAT TTC CAG CTT GGT CCC | TCT AGA GTT AAC GAT ATC AA CGT | TTA | T | CTA | A | TCA | | GCA AGA GAT GGA GGC TTG

Heavy Chain

Framework 4 ← | Stop | Stop | Stop | Framework 4 →

TGA GGT TCC TTG ACC CCA | CTG CAG AGT ACT AGG CCT CT GAG | CTA | C | TCA | G | TTA | | GGT GAT TGA GTA GCC AGT

FIG. 2

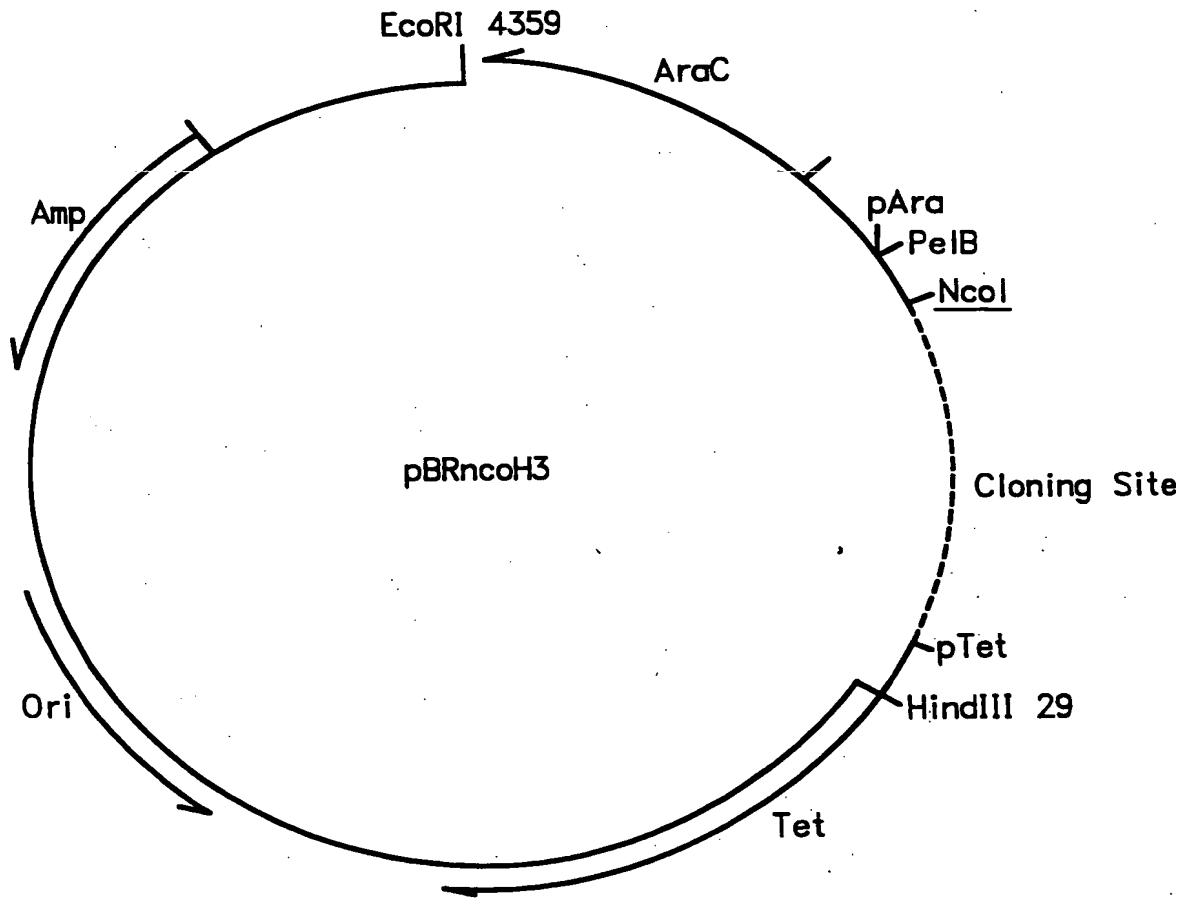

EcoRI 4359
AraC
pAra
PelB
NcoI
Cloning Site
pTet
HindIII 29
Tet
Ori
Amp
pBRncoH3

FIG. 3

AraCpBAD insert as subcloned into 14F8 to generate the pBRncoH3 cloning vector

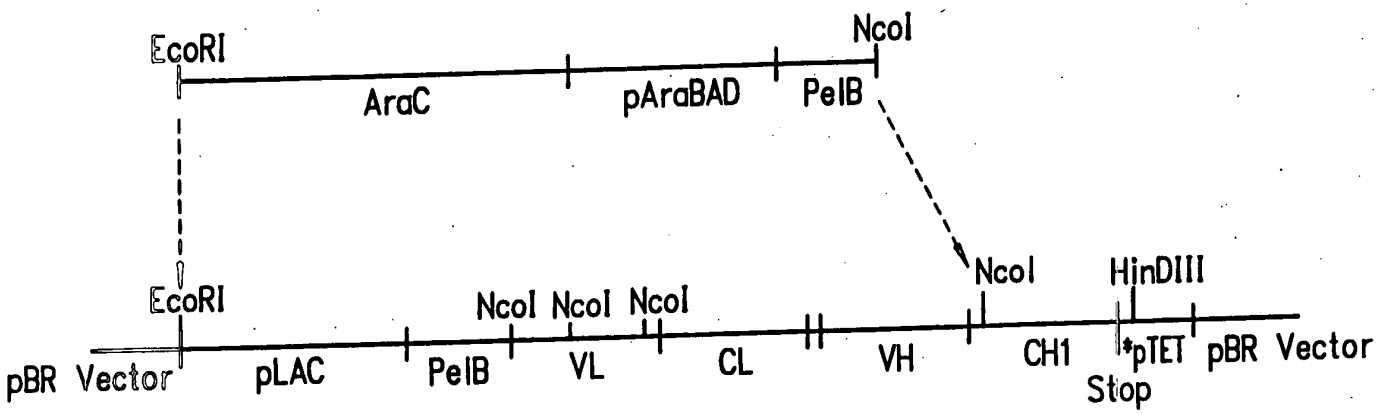
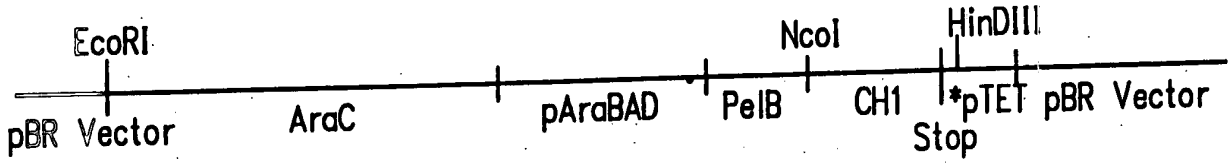


FIG. 4A

pBRncoH3 cloning vector



* represents 19 base pairs at the 5' -end of the tetracycline promoter removed by HinDIII digestion

FIG. 4B

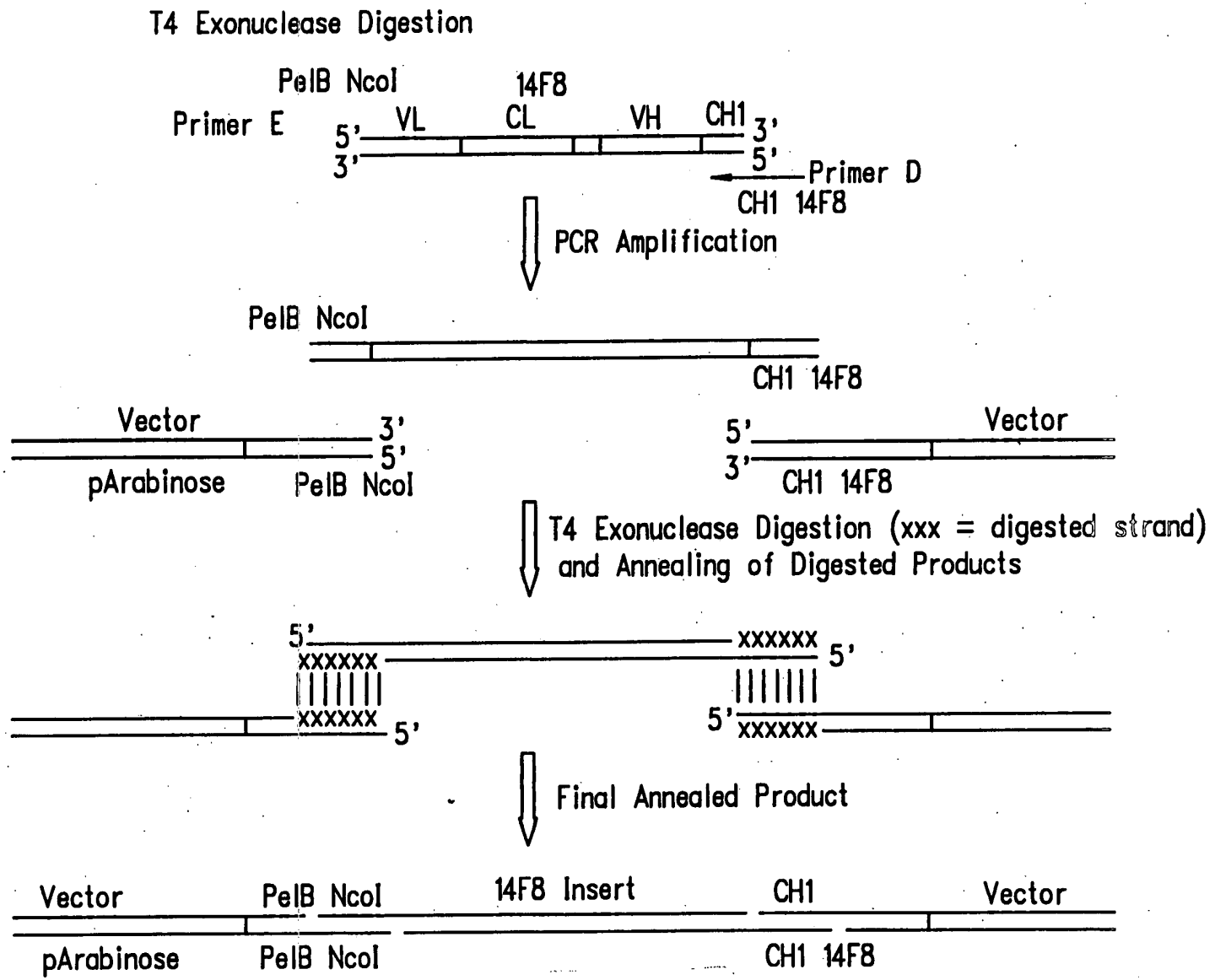


FIG. 5

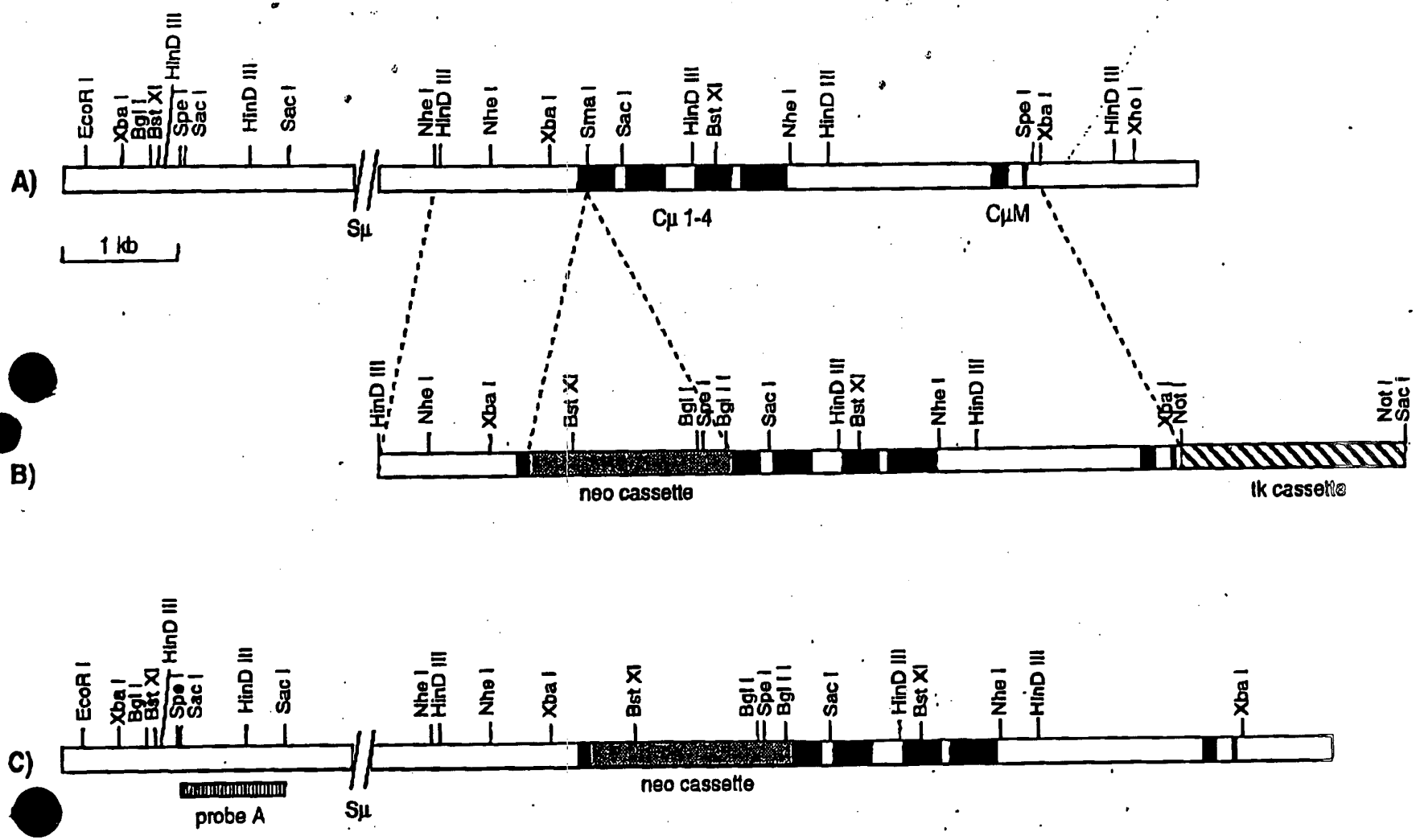


Fig. 6 Targeted insertion of a neo cassette into the Sma I site of the μ1 exon. A) Schematic diagram of the genomic structure of the μ locus. The filled boxes represent the μ exons. B) Schematic diagram of the CmD targeting vector. The dotted lines denote those genomic μ sequences included in the construct. Plasmid sequences are not shown. C) Schematic diagram of the targeted μ locus in which the neo cassette has been inserted into μ1. The box at the right shows those RFLP's diagnostic of homologous recombination between the targeting construct and the μ locus. The RFLP's were detected by Southern blot hybridization using probe A, the 915 bp Sac I fragment shown in diagram C.

Expected Fragment Sizes (kb) using Probe A		
Restriction Digest	Fragment Length wild type	mutant
Bgl I	15.7	7.7
Bst XI	7.3	6.6
Spe I	9.9	7.6
Eco RI	12.5	14.3

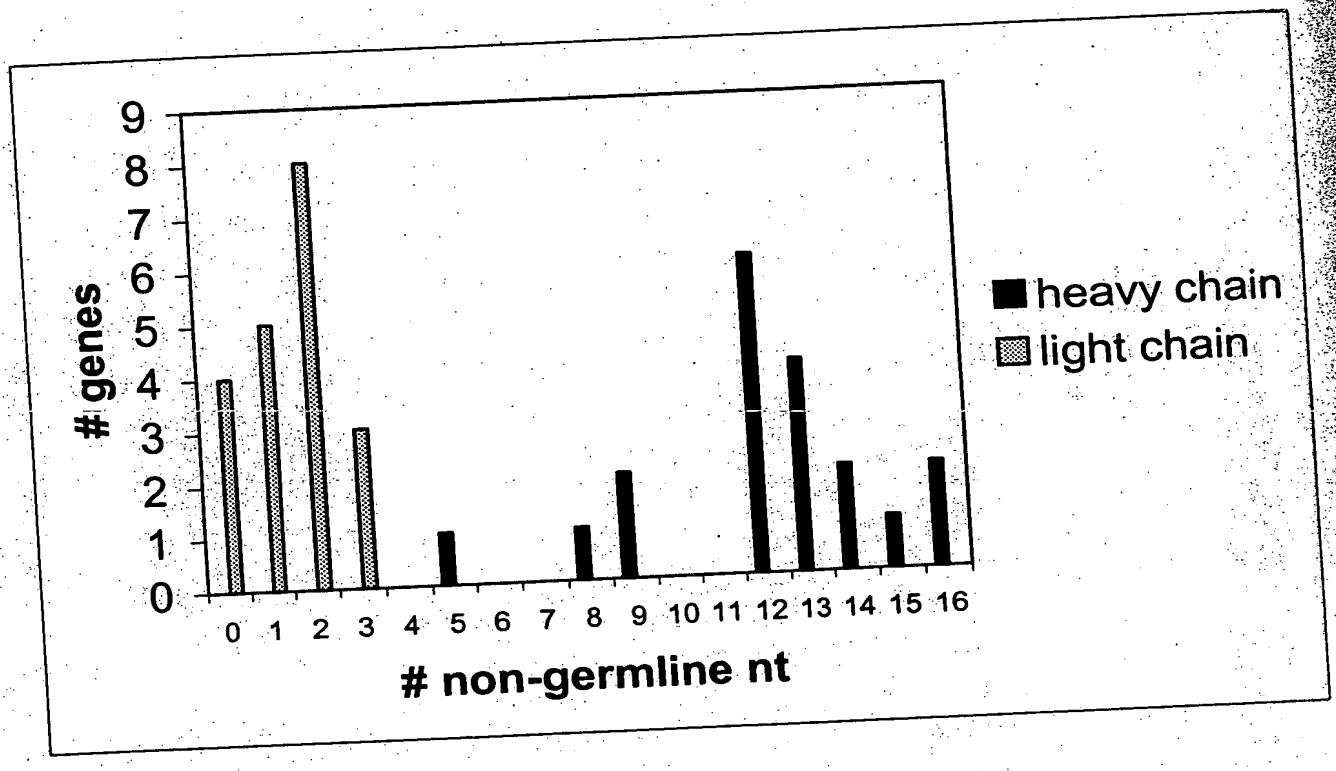

9
8
7
6
5
4
3
2
1
0
genes
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
non-germline nt
heavy chain
light chain

Figure 7.